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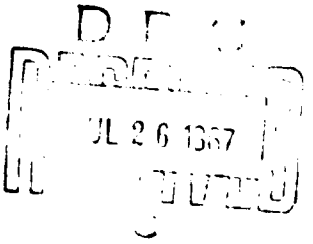

Experimental Epidemiology of Coccidioidomycosis: I. Epizootiology of Naturally Exposed Monkeys and Dogs*

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ABSTRACT

Animals from constant populations of monkeys and dogs (24 of each divided among 3 exposure sites) housed for 1 year in the open in a known endemic area for coccidioidomycosis (Tucson, Arizona) were removed – upon contracting infection with Coccidioides immitis *– to air-conditioned quarters for further observation and were immediately replaced at the exposure sites with other susceptible animals. Periodic soil and air samples were obtained, and appropriate climatic data were recorded throughout the one-year period. Clinical and laboratory observations were continuously recorded for all animals, and complete necropsies were performed at the termination of the experiment. Approximately 15% (5/34) of the monkeys (housed 26 inches above ground) and 58% (29/50) of the dogs (allowed free run of 30 x 40 ft. pens) became infected during the 1-year period, the majority (25/34) during the cooler months (November through March).*

Comparison of the pathogenesis of the disease in the naturally-infected monkeys with that in experimentally-infected monkeys indicated a natural, air-borne, infectious dose of probably less than 10 arthrospores. The infection rate, as well as the extent of disease, in the naturally-infected dogs was greater than in either the naturally-infected monkeys or the experimentally-infected dogs, and was attributed to their contact with the ground. The lack of mortality in the naturally-infected animals of either species and the minimal clinical signs and symptoms in the infected dogs indicated very low natural infectious doses of C. immitis. *The ecological and climatic parameters of this study were similar to those of other studies in the same general area.*

INTRODUCTION

THE ENDEMIC AREAS of coccidioidomycosis and the climatic and geophysical conditions necessary for growth of *Coccidioides immitis* in soil in these areas have been firmly established. Among the major contributions to these findings have been the very thorough epidemiological studies of Dr. C. E. Smith and his associates (Smith *et al.*, 1946), correlating rainfall and dry, dusty atmospheric conditions with seasonal morbidity rates; Hugenholtz's study of the optimal climatic factors for growth of the organism in the soil (Hugenholtz, 1957); the extensive studies of Dr. Roger Egeberg and co-workers, associating salt content of the soil at various seasons with optimal growth conditions for *C. immitis* (Egeberg, 1962); and a demonstration of the close association of the boundaries of the Lower Sonoran Life Zone with those of the known endemic areas, by Dr. Keith Maddy (Maddy, 1957).

Although infections with *C. immitis* result chiefly from inhalation of arthrospores picked up by wind from the soil, it is extremely difficult to isolate the organism from air samples. Consequently, the size of the infectious dose received in nature remains a matter of conjecture. The purpose of this study was an attempt to determine the approximate dose received by man in natural infections, by the use of laboratory animals as "biological air-samplers."

We have at our disposal a large amount of data on the pathogenesis of coccidioidomycosis in monkeys and dogs (Lowe *et al.*, 1959; Blundell *et al.*, 1961; Converse *et al.*, 1962; Castleberry *et al.*, 1962; and Sinski *et al.*, 1962) exposed to graded respiratory doses of *C. immitis* of from 10 to 80,000 arthrospores. In our experience, the dog was equally as susceptible to the disease as the monkey, but more resistant to its effects, through its ability to maintain a blood supply to the lesions for a longer period of time and by a faster and more prolific collagen response. It was postulated that the monkey was most susceptible to the ravages of the disease; the dog least affected; with man lying somewhere on the scale between the species, probably much closer to the dog than to the monkey. It seemed logical that a comparison of the pathogenesis (clinical and laboratory observations) of the disease in animals exposed in the open, in an endemic area, with that of animals receiving known experimental doses might lead to a valid estimation of the infectious dose received by man in nature.

*In conducting the research reported herein, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

MATERIALS AND METHODS

SELECTION OF EXPOSURE AREA

The Tucson area in southern Arizona, lying in the heart of the endemic area for coccidioidomycosis, was chosen as the exposure site. The infectivity for man in this area approaches 70% in long-time residents, and for cattle is closer to 80% (Maddy *et al.*, 1960a). Dr. Raymond E. Reed of the Animal Pathology Department of the University of Arizona agreed to supervise this study. The facilities of the College of Agriculture of the University of Arizona were available for the project.

PHYSICAL SET-UP OF EXPOSURE SITES

Three chain-link, fenced-in areas, approximately 100 ft. apart, were arranged in a shallow arc, affording each pen exposure to the prevailing wind (Figs. 1 and 2). The pens were located at the University's Casa Grande Farm in the Santa Cruz River basin (a venturi-like geographical site, where local hills funnel the prevailing winds through the area). This farm contains feed-lots, in which practically all cattle imported from non-endemic areas eventually become infected with *C. immitis* (Maddy *et al.*, 1960b).

In each of the three enclosures (Fig. 3), eight dogs were allowed free run of the area (30 x 40 ft.), and eight monkeys *(Macaca mulatta)* were confined in open cages (26 inches above ground) under appropriate shelter (Fig. 4). All animals remained at the open exposure sites for a period exceeding one year, unless they became infected with *C. immitis*, at which time they were immediately removed to air-conditioned quarters at the University's Campbell Avenue Farm, for further clinical laboratory studies, and were replaced at the exposure site with reserve, susceptible animals.

PROCEDURES FOR DETERMINATION OF INFECTION

Two dogs and two monkeys from each pen were subjected to coccioidin dermal sensitivity tests, immunodiffusion precipitin tests (Ray and Kadull, 1964), and thoracic radiographs each week, thus providing a population observation turnover once every four weeks. Tests were immediately repeated on all animals each

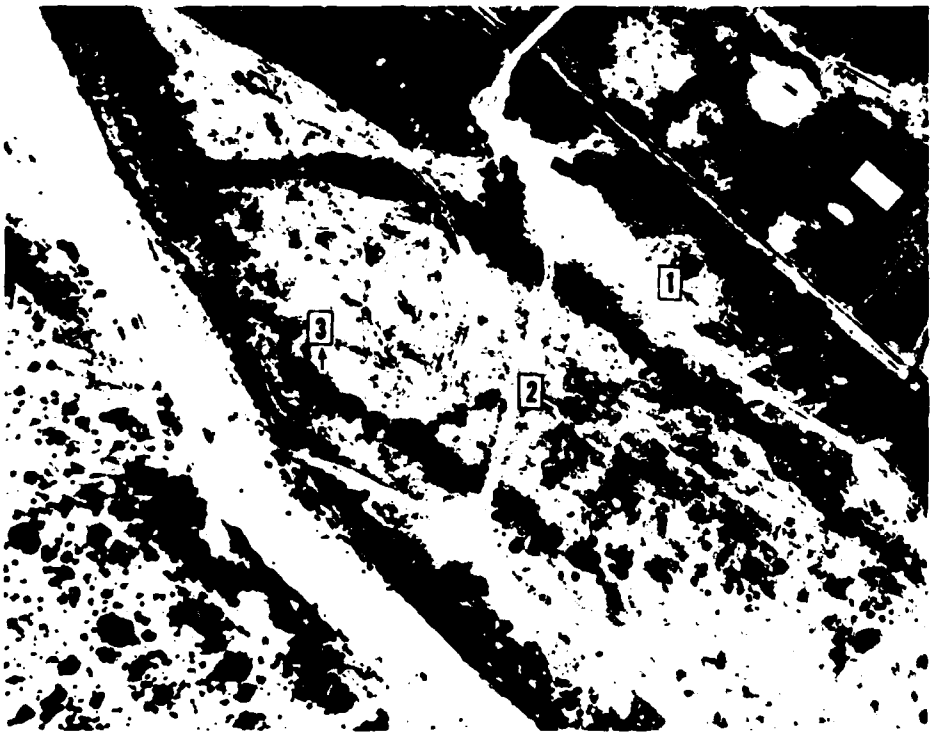

Figure 1. Air view of Casa Grande exposure area. The three exposure sites (location of pens) are indicated by arrows. Note dry bed of Santa Cruz River.

Figure 2. View of same area as in Figure 1. Note Santa Cruz River meandering through center of photograph. Exposure pens may be seen in upper left, near horizon.

time an infection was noted. All animals were critically observed, daily, for clinical signs of infection.

Controls for the study consisted of 10 monkeys and 8 dogs, inoculated intratracheally with either 10 or 100 *C. immitis* arthrospores from a culture isolated from the area under study. One normal monkey and two normal dogs were observed along with the inoculated animals.

METEOROLOGICAL DATA

Records of climatic factors such as maximum and minimum temperature, relative humidity, wind speed and direction, and rainfall were recorded daily.

SOIL AND AIR SAMPLING

Four to eight surface and subsurface soil samples were collected, bimonthly, in and around the exposure pens. These were plated directly on Mycobiotic agar, and injected (1 10 dilution of soil suspension) into six mice per sample (intranasally and intraperitoneally), for recovery of *C. immitis*.

Mycobiotic agar plates were exposed, daily, in and around the exposure pens, for recovery of *C. immitis* from the air.

PATHOLOGY STUDIES

At termination of the experiment (approximately 52-54 weeks), all animals were subjected to complete necropsies, gross pathology recorded, impression smears and cultures made from the lungs and any suspicious lesions, and histological sections of all tissues stained with routine and specific fungal stains.

RESULTS AND DISCUSSION

THE METEOROLOGICAL FACTORS (Table 1) in the area under study (throughout the period of October, 1963, to October, 1964) were similar to those of former studies in the same general region. Maddy (1957) and Hugenholtz (1957) reported mean July temperatures of 80° to 90° C. over a period of years at various locations, as compared with 88° C. for the present study, with mean maximum, and peak temperatures of 105° and 110° C, respectively, as compared with 101° and 110°C. in our study. Mean January temperatures of 50° to 55°C. in the former studies were in accord with the 47°C. at Casa Grande Farm. The average yearly rainfall of 9 inches reported by Maddy, and 6 to 10 inches by Hugenholtz, compared favorably with the 12.5 inches recorded by us. The only factors which might have affected this study adversely were mean winter temperatures about 10 degrees below normal, with a very late, cold spring.

Prevailing winds came mostly from the west through the north quadrant, with gusts sometimes as high as 35 mph. All attempts to isolate *C. immitis* from the air

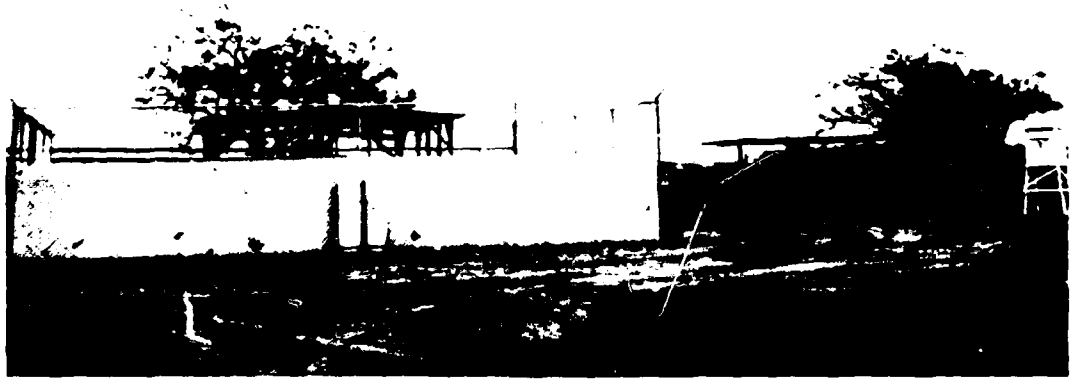

Figure 3. View of one of the three exposure pens. The solid appearing structure extending up the fence from the ground is made of louvered aluminum and permits the entry of wind and dust. The roofed structure visible inside the fence is the monkey shelter.

Figure 4. Close-up view of monkey shelter. Note automatic bottled gas heater extending up through center of cage battery which furnishes heat during severe weather conditions. The battery consists of 4 cages (housing 2 monkeys each) completely open on 4 sides, partially open on the 5th side. Note one of the oil-drum dog shelters in the background. The dogs have free run of the fenced area (30 x 40 ft.)

TABLE I

COMPARISON OF WEATHER CONDITIONS IN THREE EPIDEMIOLOGICAL STUDIES

Observation	Maddy[a]	Hugenholtz[b]	The Present Study
Mean July Temperature	90	80-90	88
Mean Maximum Temperature	105		101
Peak Temperature		110	110
Mean January Temperature	50	50-55	47
Minimum Temperature			14
Rainfall (inches per year)	5-20 (Avg. 9)	6-10	12.5

[a] – Maddy, 1957.
[b] – Hugenholtz, 1957.

met with failure, although two soil samples collected in August, 1964, and two in October, 1964, were positive for the fungus.

Five of 34 monkeys and 29 of 50 dogs became infected during the 12-month exposure period (Fig. 5 and Table II), the majority (25 34) during the cooler months of November through March.

Each increase in infections occurred after a cycle of rainfall followed by a comparatively dry period, as had been previously reported. However, the low infectivity rate (unexpected) during July and August may have been due to excessive rainfall (4.5 and 2.5 inches, respectively).

Only three of the five infected monkeys demonstrated positive serological titers and only two monkeys exhibited histological lung changes indicative of coccidioidomycosis (and these of a very minor nature). Lung cultures of all monkeys were negative for *C. immitis*. The five infected monkeys remained in good health during the 12-month period, showing no clinical symptoms of

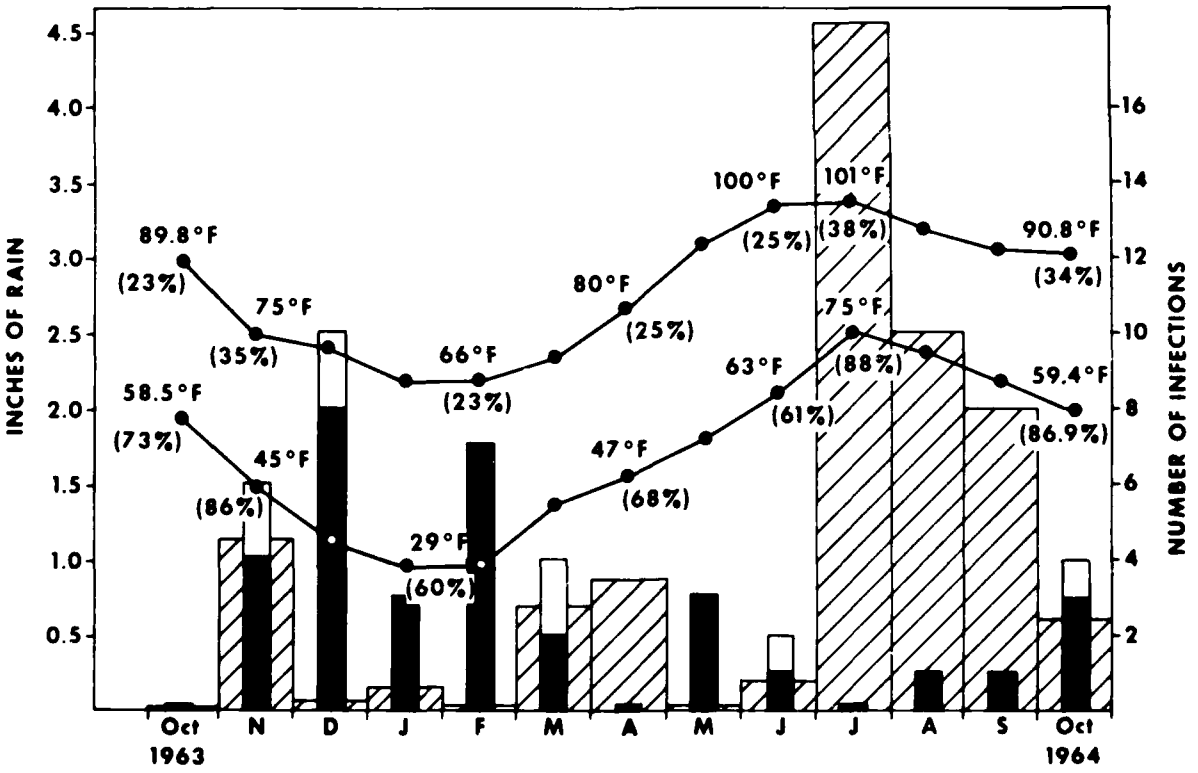

Figure 5 Correlation of weather conditions with infection with **C. immitis**. Cross-hatched bar indicates rainfall in inches; solid bar, number of infections, and solid lines mean maximum and minimum temperatures for each month. The figures in parentheses represent the relative humidity at the temperature indicated. The open portion at the top of some of the solid bars indicates suspected but unproven infections.

TABLE II

NATURAL INFECTIONS IN MONKEYS AND DOGS

Animal	Number Exposed	Number Infected	Number Positive Skin Test	Number Positive Serology	Number Showing Patho-logical Changes	Number Positive Lung Cultures	Average Days to Infection
Dog	50	29	20	22	18	3	139[a] (10-365)
Monkey	34	5	5	3	2 Very minor	0	88 (42-121)

[a] — 10-20 days in peak infection period (Jan. to Mar.) Figures in parentheses indicate span.

TABLE III

COMPARISON OF NATURALLY-EXPOSED INFECTED ANIMALS WITH EXPERIMENTALLY-INFECTED ANIMALS IN FORMER STUDIES

Monkey		
Dose[a]	Mean[b] Maximum Titer	% Mortality
Natural Exposure (Unknown)	1:8 (Neg. to 1:64)	0
10	1:128 (1:64 to 1:256)	40
50-100	±1:256 (1:128 to 1:256)	30
300	1:512 (1:128 to 1:1024)	40
Dog		
Dose[c]	Mean Maximum Titer	% Mortality
Natural Exposure (Unknown)	1:16 (Neg. to 1:512)	0
10	—[d]	10
100	—	20
1000	—	47

[a] — Numbers refer to aerosol arthrospore doses (experimental), Lowe et al., 1959; Converse et al., 1962.

[b] — Immunodiffusion precipitin test. Numbers in parentheses indicate spread.

[c] — Numbers refer to intratracheal arthrospore dose (Reed, Raymond E. — personal communication).

[d] — Test not made.

disease; their serological titers were comparatively low (mean maximum of 1-8, with a range of negative to 1-64); and very little evidence of infection was noted in the X-Rays.

A comparison of these data with that of experimentally-infected monkeys (respiratory doses of from 10 to 300 arthrospores) in several former studies (Table III) indicated a very small natural infectious dose (probably in the 10, or less than 10, arthrospore range). This undoubtedly represented air-borne infection, since the monkeys were housed several feet above ground level.

The infection rate, as well as the extent of the disease, was much greater in the naturally-infected dogs than in the naturally-infected monkeys, suggesting a larger infectious dose in the dogs. This was attributed to their contact with the soil, their habit of constantly digging and fighting, with the consequent stirring up of dust, and particularly the fact that the majority of dog infections (19/29) occurred in *one* of the three exposure pens; all of which indicated that the dogs were becoming infected from contact with the ground, rather than from *normal* air-borne aerosols.

Of the 29 naturally-infected dogs (Table II), 20 exhibited positive skin tests, 22 positive precipitin titers, 18 showed histological lung changes due to *C. immitis*, and 3 had *C. immitis* isolated from the lungs by culture. Four of the 29 were diagnosed by histological studies only; all clinical and laboratory tests being negative.

No deaths resulting from coccidioidomycosis occurred in either species. Clinical signs and symptoms of the disease were lacking in the monkeys, and relatively mild in the dogs, all of which indicated low infectious doses received by the naturally-exposed animals (probably less than 10 in the monkeys and less than 100 in the dogs).

ACKNOWLEDGEMENTS

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